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Characterization of an ATP Translocase Identified in the Destructive Plant Pathogen "*Candidatus* Liberibacter asiaticus"▽

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ATP/ADP translocases transport ATP across a lipid bilayer, which is normally impermeable to this molecule due to its size and charge. These transport proteins appear to be unique to mitochondria, plant plastids, and obligate intracellular bacteria. All bacterial ATP/ADP translocases characterized thus far have been found in endosymbionts of protozoa or pathogens of higher-order animals, including humans. A putative ATP/ADP translocase was uncovered during the genomic sequencing of the intracellular plant pathogen "*Candidatus* Liberibacter asiaticus," the causal agent of citrus huanglongbing. Bioinformatic analysis of the protein revealed 12 transmembrane helices and predicted an isoelectric point of 9.4, both of which are characteristic of this family of proteins. The "*Ca.* Liberibacter asiaticus" gene (*nttA*) encoding the translocase was subsequently expressed in *Escherichia coli* and shown to enable *E. coli* to import ATP directly into the cell. Competition assays with the heterologous *E. coli* system demonstrated that the translocase was highly specific for ATP and ADP but that other nucleotides, if present in high concentrations, could also be taken up and/or block the ability of the translocase to import ATP. In addition, a protein homologous to NttA was identified in "*Ca.* Liberibacter solanacearum," the bacterium associated with potato zebra chip disease. This is the first reported characterization of an ATP translocase from "*Ca.* Liberibacter asiaticus," indicating that some intracellular bacteria of plants also have the potential to import ATP directly from their environment.

Citrus huanglongbing (HLB), also known as citrus greening, is a disease of citrus that was first reported in China in the early 20th century (33) and identified in the United States in August 2005 in South Florida (22). As it spread rapidly across Florida, HLB has caused substantial economic losses to the citrus industry, and now other citrus-producing states may be in danger as well. The effects of this disease range from mild to severe and include symptoms such as yellow shoots, blotchy mottles on leaves, vein yellowing and corking, lopsided fruit with aborted seeds, early fruit dropping, and limb dieback, which can ultimately lead to the total loss of the infected tree. The disease has been associated with three species of bacteria known as "*Candidatus* Liberibacter" species. Each of the three "*Ca.* Liberibacter" species was discovered and named based on its presumptive origin, with "*Ca.* Liberibacter asiaticus" being found in Asia, "*Ca*. Liberibacter africanus" in Africa (13), and "*Ca*. Liberibacter americanus" in South America (24). A fourth species, known as "*Ca.* Liberibacter solanacearum," is genetically related, although it is not naturally associated with HLB in citrus plants (16). "*Ca.* Liberibacter solanacearum" is associated with the emerging zebra chip disease of potatoes and tomatoes (15). "*Ca.* Liberibacter" species are Gram-negative, fastidious alphaproteobacteria (13) that reside in the sieve tube elements of infected plants (23). The same bacteria found in citrus plants have also been found in two phloem-feeding insects, the Asian citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*), which act as vectors for the disease (for recent reviews, see references 3 and 9). Since insects that carry the pathogen do not have a shortened life span or other adverse effects (12), "*Ca.* Liberibacter" is thought to act more as an endosymbiont than as a pathogen in insects. There is no known cure for HLB, and current management strategies include elimination of infected trees and methods aimed at vector control. Because of the rapid spread and devastating consequences of infection with "*Ca.* Liberibacter," understanding this obligate intracellular pathogen will be critical for the survival of the citrus industry.

Recently, the complete genome sequence of "*Ca.* Liberibacter asiaticus" was obtained via metagenomics (5). Within this "*Ca.* Liberibacter asiaticus" genome, an open reading frame encoding a putative ATP/ADP translocase was found. Translocases are enzymes that aid in the transport of molecules, in this case adenosine phosphate, across a cell membrane. These adenylate transporters can be placed into one of three groups based upon where they reside. The first group was discovered in mitochondria and is involved in transporting the ATP synthesized in the mitochondrial matrix to the cytosol of the cell (28). The second type of transporter is found in plant plastids (19, 21, 31). In contrast to the mitochondrial transporters, which transport ATP to the cytosol, this set of transporters import ATP from the cytosol. Their function is to provide the stroma with a supply of cytosolic ATP in order to facilitate many of the anabolic reactions that take place there. The third set of transporters was originally discovered in the obligate intracellular bacterium *Rickettsia prowazekii* (30). Similar to their plastid counterparts, these transporters import ATP from the host cell's cytosol and translocate it into the bacterial cell. Bacteria that posses this enzyme can act as "energy parasites" and import ATP directly from their hosts.

Since its discovery in *Rickettsia*, the ATP/ADP translocase has been identified in other obligate intracellular parasites of

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animals, such as *Chlamydia psittaci* and *Lawsonia intracellularis* (11, 20), in addition to some protist endosymbionts, such as *Caedibacter caryophilus* and "*Protochlamydia amoebophila*" (4, 10). Analyses of the translocase proteins in these bacteria have demonstrated that certain translocase homologs can be used by the cell to import nucleotides other than ATP (2, 4, 10, 26), and thus, the family of proteins has come to be known more generally as nucleotide transporters. In spite of all of the previous research in this area, an ATP/ADP translocase from a bacterial plant pathogen has yet to be characterized. Here, we present the first characterization of a nucleotide transport protein (NttA) from the obligate intracellular plant pathogen "*Ca.* Liberibacter asiaticus."

MATERIALS AND METHODS

Bioinformatic analysis of the predicted translocase. The percent identity of two related proteins was determined using the ClustalW algorithm in the Align X module of Vector NTI (Invitrogen). The ProtParam tool on the ExPASy server (http://www.expasy.ch/tools/) was used to predict both the molecular weight and the isoelectric point (pI) of a protein (8). Protein sequences from *R. prowazekii* (corresponding to accession no. AAA26382), *Chlamydia trachomatis* (accession no. CAB39534), *L. intracellularis* (accession no. YP_594385), and *Arabidopsis thaliana* (accession no. Z49227) were all analyzed in this manner. The ConPred II server (http://bioinfo.si.hirosaki-u.ac.jp/~ConPred2/) was used to predict the transmembrane topology for NttA (1).

Bacterial strains and media. *Escherichia coli* Top10 cells (Invitrogen) were used as a host for plasmid construction, while strain BL21 Star(DE3) (Invitrogen) was used for subsequent translocase expression. *E. coli* was grown either in Luria-Bertani (LB) broth or on LB agar plates supplemented with 50 μg/ml of ampicillin at 37°C.

Isolation of "*Ca.* Liberibacter asiaticus" genomic DNA from "*Ca.* Liberibacter asiaticus"-infected psyllids. "*Ca.* Liberibacter asiaticus" genomic DNA was isolated from psyllids (*D. citri*) collected from citrus fields in Fort Pierce, FL. Psyllids were homogenized in PBS-BSA buffer (0.1% bovine serum albumin in phosphate-buffered saline, pH 7.2) and centrifuged at 5,000 × *g* for 5 min at 4°C to remove debris. The supernatant was then centrifuged at 10,000 × *g* for 10 min at 4°C to pellet the bacterial cells. The pellet was resuspended in PBS-BSA, and both the low- and high-speed spins were repeated as described above. The final pellet was resuspended in a mixture of 24 μl PBS-BSA, 3 μl DNase buffer, and 3 μl DNase I, and the suspension was incubated at 37°C for 1 h before the addition of 3 μl stop solution. Antibodies were then used to aid in the isolation of the "*Ca.* Liberibacter asiaticus" bacterial cells. These polyclonal antibodies, rabbit anti-OMP1 and rabbit anti-OMP2, were produced against two peptides (CIEGNDQSYDSVIRRE and CGIPLRHREGDKIQQF, respectively) within the "*Ca.* Liberibacter asiaticus" outer membrane proteins and affinity purified using the peptides mentioned above (Antagene, Inc.). Both anti-OMP1 and anti-OMP2 antibodies were added to the cell suspension and gently mixed for 10 min at 2 to 8°C. Cells were washed in PBS-BSA to remove excess antibody, centrifuged at 5,000 × *g*, and resuspended in 1 ml PBS-BSA. The "*Ca.* Liberibacter asiaticus" cells were isolated using 40 μl of M-280 sheep anti-rabbit IgG Dynabeads according to the instructions of the manufacturer (Dynal Biotech), with the final target cell-Dynabead mixture resuspended in 9 μl PBS-BSA. The amount of DNA was increased using multiple-displacement amplification (MDA).

MDA of "*Ca.* Liberibacter asiaticus" genomic DNA. The DNA isolation and MDA reaction were performed with 3 μl of the suspension of "*Ca.* Liberibacter asiaticus" cells isolated from psyllids by using the REPLI-g minikit according to the protocol of the manufacturer (Qiagen).

DNA manipulation. Initial PCR amplification of the translocase gene was performed with Platinum *Taq* high-fidelity polymerase (Invitrogen), while all subsequent PCR amplifications were performed with *Taq* DNA polymerase (New England Biolabs). DNA sequencing was done by the U.S. Horticulture Research Laboratory Core Facility using BigDye Terminator version 3.1 and the 3730xl DNA analyzer (Applied Biosystems). Plasmid DNA was isolated from *E. coli* cultures using the QIAprep Spin Miniprep kit (Qiagen).

Construction of pCMV203, the *nttA* expression plasmid. A plasmid containing *nttA*, the "*Ca.* Liberibacter asiaticus" translocase gene, was constructed by amplifying the gene from genomic DNA isolated from "*Ca.* Liberibacter asiaticus"-infected psyllids with the gene-specific primers ATPTrans-F2 (5′-gcggtctagaATGTCGGAGGCGAAG-3′) and ATPTrans-R2 (5′-gcggctcgagTTCTTTACTAATAAGCTGAGTAT-3′; lowercase letters indicate restriction sites) and Platinum *Taq* high-fidelity polymerase (Invitrogen) using the following PCR conditions: 2 min of denaturation at 94°C, followed by 36 cycles of 94°C for 30 s, 56°C for 30 s, and 68°C for 2 min. The product was digested with XhoI and XbaI (New England Biolabs) at the engineered restriction sites that were added to the primers, which are noted in the primer sequences above. The vector Champion pET303/CT-His (Invitrogen) was also digested with XhoI-XbaI, and both the 5.4-kb vector and the amplified 1.4-kb DNA fragment were purified on an agarose gel with the QIAquick gel extraction kit (Qiagen). T4 DNA ligase (New England Biolabs) was used to ligate the purified products, producing pCMV203. The clone was verified via DNA sequencing, and BL21 Star(DE3) was transformed with the construct.

Expression of NttA in *E. coli*. The bacterial strains BL21 Star(DE3)(pET303) and BL21 Star(DE3)(pCMV203) were grown with shaking to an optical density at 600 nm (OD_{600} [A_{600}]) of ~0.45 in LB medium supplemented with 50 μg/ml of ampicillin at 37°C. Expression of NttA was induced through the addition of 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) to the medium, and cultures were grown for an additional hour.

Reverse transcription-PCR. Total RNA was extracted from samples using the Trizol Max bacterial RNA isolation kit according to the protocol of the manufacturer (Invitrogen). Extracted RNA was treated with DNase I (Invitrogen) to remove any contaminating DNA before being used in reverse transcription reactions. First-strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen), 0.5 μg of RNA, and the gene-specific primers ATPTrans-F2/ATPTrans-R2. PCR was then performed with a 20-μl volume containing 1 μl of the cDNA reaction mixture, 1× Premix D (Epicentre), the primer pair ATPTrans-F2/ATPTrans-R2, distilled water, and *Taq* DNA polymerase (New England Biolabs). The PCR profile consisted of 2 min of denaturation at 94°C, followed by 36 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min. The final PCR step was an incubation period of 5 min at 72°C before the reaction mixture was held at 4°C. Plasmid DNA extracted from the Top10(pCMV203) strain was used as a positive control for the PCR. PCR products were electrophoresed on 1% agarose gels and visualized with ethidium bromide staining.

Uptake assay with radiolabeled ATP. Uptake assays were performed as described previously by Winkler (29) with some modifications. Cultures of BL21 Star(DE3)(pET303) and BL21 Star(DE3)(pCMV203) were grown and divided into two portions. Half of each culture was kept as a control without induction, and the other half was induced as described above (see "Expression of NttA in *E. coli*"). Cells were collected after the 1-h outgrowth period via centrifugation, washed, and resuspended in 50 mM phosphate-buffered saline (PBS), pH 7.5, to an OD_{600} of ~1.0. Then 400 μl of washed cells was added to 300 μl of PBS containing 0.3 μl [α-^{32}P]ATP (10 mCi/ml, with a specific activity of 3,000 Ci/mmol), and the mixture was incubated at 30°C. Samples of 150 μl were taken at the time points indicated in the figures and centrifuged. Cells were washed twice in ice-cold PBS and resuspended in a solution of PBS and scintillation fluid (1:1) before being measured on a Wallac TriLux 1450 microbeta scintillation counter.

Competition assay with nonradiolabeled nucleotides. Cultures were grown, induced, and resuspended in PBS to an OD_{600} of ~1.0 as described above. Subsequently, 800 μl of cells was added to 600 μl of PBS containing 0.6 μl [α-^{32}P]ATP (10 mCi/ml, with a specific activity of 3,000 Ci/mmol). Cells were aliquoted into 150-μl samples, and unlabeled nucleotides were added to a final concentration of 0.23 or 2.3 mM. Samples were incubated at 30°C for 30 min, washed, and measured as described above.

"*Ca.* Liberibacter solanacearum" genomic sequences for a putative ATP/ADP translocase. The draft genome sequence of "*Ca.* Liberibacter solanacearum" (H. Lin, unpublished data) was searched for genes encoding potential protein homologs by using the "*Ca.* Liberibacter asiaticus" translocase sequence.

RESULTS

The "*Ca.* Liberibacter asiaticus" genome contains a single ATP/ADP translocase gene with properties similar to those of other known translocase genes. Annotation of the completed genome of "*Ca.* Liberibacter asiaticus" revealed the presence of a single gene predicted to encode an ATP/ADP translocase (corresponding to accession no. GU011685) (5). The *nttA* gene encodes a protein 469 amino acids in length that is 39 and 47% identical to the ATP/ADP translocases from *C. trachomatis* (accession no. CAB39534) and *R. prowazekii* (accession no.

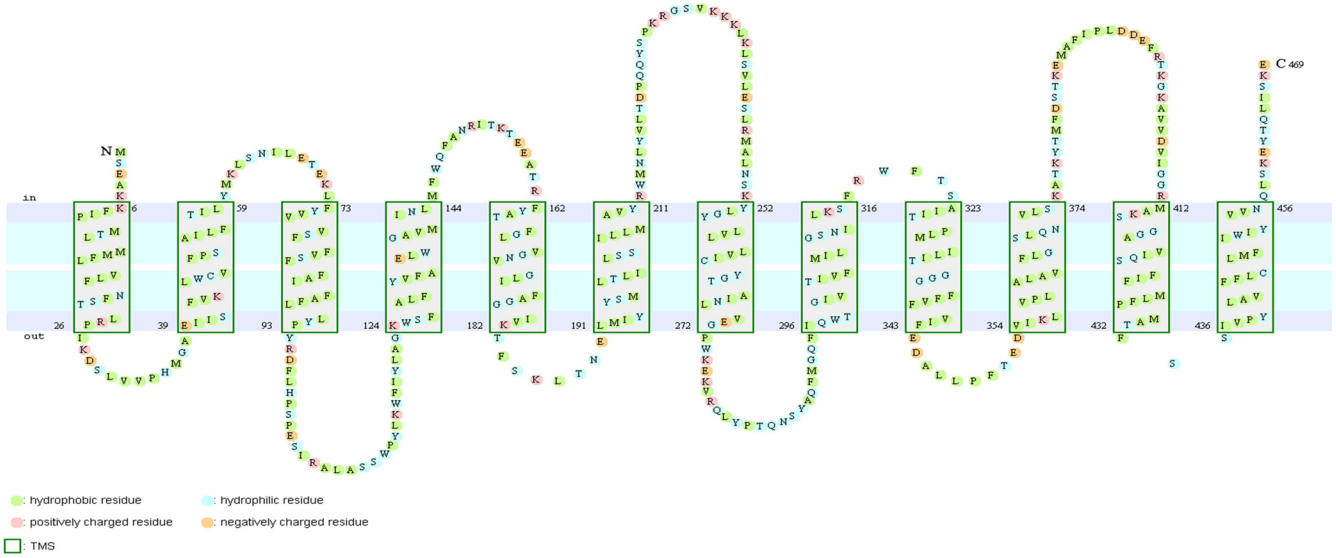

FIG. 1. Transmembrane topology of NttA as predicted by ConPred II (1) The putative translocase found in "*Ca.* Liberibacter asiaticus" contains 12 transmembrane (TMS) helices (green boxes). Numbers correspond to amino acid positions. This structure is consistent with those of other known ATP/ADP translocases characterized to date.

AAA26382), respectively (data not shown). NttA was predicted to have a molecular mass of 54 kDa and a pI of 9.4. This prediction is consistent with the molecular masses and pIs of other known translocases, which range from 57 to 69 kDa and from 9.1 to 9.8, respectively (data not shown). In addition, NttA was predicted to contain 12 transmembrane helices (Fig. 1), a feature that is also characteristic of this family of proteins.

Expression of NttA in the heterologous host *E. coli*. Due to the inability to genetically manipulate "*Ca.* Liberibacter asiaticus" at this time, a plasmid (pCMV203) was constructed in which *nttA* was expressed from the inducible T7 promoter, and *E. coli* strain BL21 Star(DE3) was subsequently transformed with the construct. BL21 Star(DE3) was also transformed with the vector alone (pET303) for use as a negative control. Since *E. coli* does not contain any genes homologous to the translocase gene, any ATP taken up by the cell can be attributed to the presence of NttA. In order to verify expression of *nttA* in the heterologous host, reverse transcription-PCR was used to determine the relative amounts of transcripts produced by each of the strains in both uninduced and induced states (Fig. 2). Only the strain containing *nttA* (pCMV203) produced detectable levels of transcripts. As expected, the *E. coli* strain transformed with pCMV203 produced a large amount of transcripts upon induction. A small amount of transcripts was also present when this transformed strain was not induced, which can be expected for this system (18). The transformed *E. coli* strains were used for all subsequent experiments described herein.

"*Ca.* Liberibacter asiaticus" encodes a functional ATP translocase. The functionality of NttA was tested by assaying the *E. coli* cultures for their abilities to import [α-^{32}P]ATP directly from the medium. The amount of ATP taken up into the cell was measured 10, 20, and 30 min after the addition of [α-^{32}P]ATP to the medium, producing a time course of the amount of ATP internalized in each strain (Fig. 3A). The amount of ATP uptake in the culture of the induced strain carrying pCMV203, which expressed *nttA* at a high level (Fig. 2, lane 9), increased from an average of 36 cpm (at time zero) to 1,423 cpm after a 30-min incubation at 30°C. The uninduced strain carrying pCMV203 showed a level of up to 150 cpm, which is still greater than the 32 to 40 cpm measured for the vector (pET303)-only strain in either the induced or the uninduced state. This uptake by the uninduced *nttA* strain (carrying pCMV203) can be attributed to the low level of expression seen without induction of the translocase (Fig. 2, lane 7). Upon induction of NttA expression, the average changes in ATP uptake after incubation times of 10, 20, and 30 min were 7.5-, 7.7-, and 9.7-fold, respectively (Fig. 3B), demonstrating that NttA was indeed functional.

NttA is highly specific for both ATP and ADP. The specificity of NttA was determined by competition assays with radiolabeled [α-^{32}P]ATP and the nonradiolabled ribonucleotides ATP, ADP, AMP, CTP, GTP, and UTP (Fig. 4). At a concentration of 0.023 mM (Fig. 4, black solid bars), ATP and ADP were able to block uptake of the radiolabeled ATP by 78 and 82%, respectively, thus indicating specificity for both of these substrates. In the presence of other nucleotides (AMP, CTP, GTP, and UTP), there was only a slight reduction in uptake, ranging between 20 and 38%. These results indicate that NttA does not have a high degree of specificity for these substrates. Interestingly, the other ribonucleotides (AMP, CTP, GTP, and UTP), if present at a concentration of 2.3 mM (Fig. 4, gray solid bars), could prevent ATP from being taken up by the translocase, although it is unclear whether this was due to specific or nonspecific inhibition. Uptake by both the uninduced (data not shown) and induced vector controls remained at background levels.

Conservation of the ATP translocase among "*Ca.* Liberibacter" species. The genome sequence of a related "*Ca.* Liberibacter" species, "*Ca.* Liberibacter solanacearum," revealed the existence of a putative ATP translocase (corresponding to accession no. GU187359) that was 85% identical

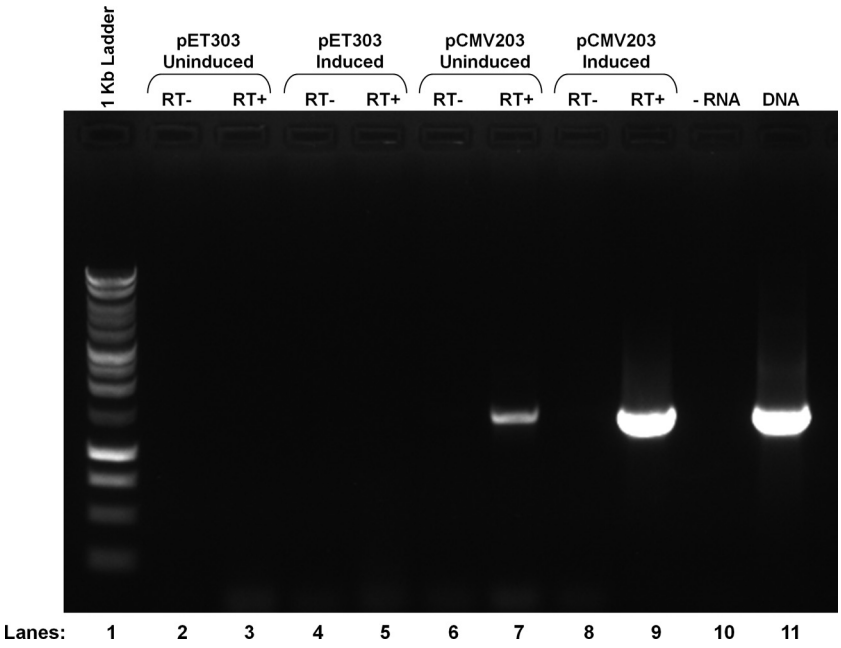

FIG. 2. Transcription of *nttA* in a heterologous host. *E. coli* BL21 Star(DE3) was transformed with a plasmid expressing *nttA* from the inducible T7 promoter, producing strain BL21 Star(DE3)(pCMV203). BL21 Star(DE3) was also transformed with the vector backbone for use as a negative control, producing strain BL21 Star(DE3)(pET303). IPTG was added to the medium to induce expression of the translocase, and the level of expression was determined using reverse transcription-PCR. Lanes: 1, 1-kb marker; 2 and 3, results from reverse transcription-PCR using RNA extracted from the uninduced vector control strain (carrying pET303) without reverse transcriptase (−RT) and with reverse transcriptase (+RT), respectively; 4 and 5, results from reverse transcription-PCR using RNA extracted from the induced pET303-containing strain without and with reverse transcriptase, respectively; 6 and 7, results from reverse transcription-PCR using RNA extracted from the uninduced *nttA* (pCMV203)-containing strain without and with reverse transcriptase, respectively; 8 and 9, results from reverse transcription-PCR using RNA extracted from the induced pCMV203-containing strain without and with reverse transcriptase, respectively; 10, results for negative control with no RNA (−RNA); and 11, results for positive control with DNA from pCMV203. The results shown are representative of data from three independent replicates.

to NttA. This protein was found by performing a BLAST search of the draft "*Ca.* Liberibacter solanacearum" genome sequence using NttA as the protein query sequence. An amino acid sequence alignment of the ATP translocase from "*Ca.* Liberibacter asiaticus" with that from "*Ca.* Liberibacter solanacearum" is shown in Fig. 5. The translocase encoded by "*Ca.* Liberibacter solanacearum" also contains 12 transmembrane domains and has a pI of 9.5.

DISCUSSION

In this study, an ATP translocase encoded by the bacterial plant pathogen "*Ca.* Liberibacter asiaticus," NttA, was expressed in *E. coli* and the protein was shown to function as an import system for ATP (Fig. 3). The ability to recapitulate the system in a heterologous host implies not only that codon usage patterns in the two bacteria are similar but also that the leader sequence was recognized and the protein was properly processed in the inner bacterial membrane of the host, suggesting that *E. coli* may serve as an adequate intermediate for future structure/function studies of obligate intracellular "*Ca*. Liberibacter" bacteria. NttA showed structural (Fig. 1) and biochemical properties similar to those of translocases from other obligate intracellular organisms, including the conservation of all four amino acids identified as important for substrate specificity in the plastidic ATP/ADP transporter from *Arabidopsis* (27). Considering that both NttA and the *Arabidopsis* translocase show specificity for ATP and that ADP acts as a competitive inhibitor for both translocase reactions (Fig. 4), conservation of these critical residues would be expected (25).

Since ATP and ADP, but not other ribonucleotides, are the preferred substrates (Fig. 4), it can be concluded that NttA provides the "*Ca.* Liberibacter asiaticus" cell with energy and not nucleotides in general. Given the reduced genome size of "*Ca.* Liberibacter asiaticus" (1.2 Mb) and other intracellular pathogens, genes that have been maintained throughout the evolutionary process are predicted to be essential and/or highly advantageous. In addition, the importance of the translocase was exemplified by its conservation in the related "*Ca.* Liberibacter" species "*Ca.* Liberibacter solanacearum" (Fig. 5). Like *Rickettsia* (32), however, "*Ca.* Liberibacter asiaticus" appears to encode a functional tricarboxylic acid cycle (5), making the need for a translocase unclear. In addition, "*Ca.* Liberibacter asiaticus" appears to carry only a single translocase gene, unlike most other bacterial species, which contain multiple isoforms of the gene (2, 10, 26). A closer examination of the metabolic pathways is necessary before the role of the protein in pathogen survival can be determined. Although an essential role of the translocase has yet to be determined, information derived from this study suggests that the capability of acquiring ATP from the host could play an important role in this obligate intracellular bacterium and that this feature may be exploited

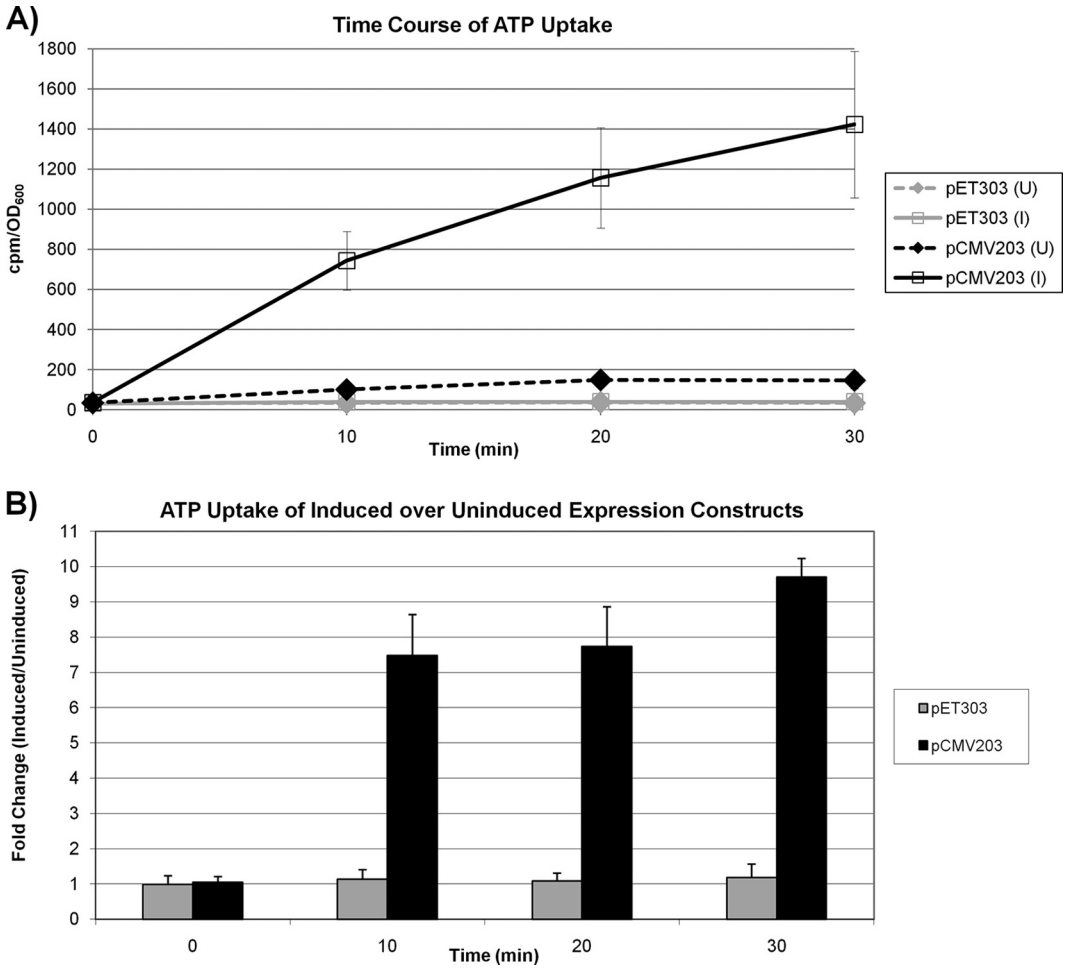

FIG. 3. Uptake of [α-^{32}P]ATP by NttA in *E. coli*. (A) A time course for the uptake of ATP by NttA was constructed using *E. coli* transformed with either a plasmid containing *nttA* (pCMV203) or the vector control (pET303). Upon reaching an OD_{600} of ~0.45, the culture volume was split and half was induced (I) with IPTG while the other half remained uninduced (U). Cultures were grown for an additional 60 min before an aliquot was removed and resuspended in PBS containing [α-^{32}P]ATP. Samples of the suspensions were taken at the indicated time points and washed to remove external [α-^{32}P]ATP, and the amount of radioactive ATP taken up by the cells (expressed as counts per minute per unit of OD_{600}) was measured. (B) The overall change (*n*-fold) at each time point was determined by dividing the [α-^{32}P]ATP uptake (expressed as counts per minute per unit of OD_{600}) in the induced samples by the [α-^{32}P]ATP uptake (expressed as counts per minute per unit of OD_{600}) in the uninduced samples. The average of results for three independent replicates is shown along with the corresponding standard deviation.

to facilitate a means for growing an axenic culture of this bacterium *in vitro*.

Stemming from the recent flurry of research pertaining to HLB symptoms and detection, the ramifications of the disease for the plant are becoming more clearly defined. Microscopic analysis of sweet oranges showed an accumulation of starch in the mesophyll parenchyma cells in plants with HLB compared to healthy controls (14). The reason for this accumulation is unknown, although it was proposed to result from a combination of the restriction of photosynthate movement by phloem plugging and the upregulation of the starch biosynthesis genes in response to infection. It is possible that the perturbation of the ATP level within a cell resulting from the import of ATP via NttA may play a role in the increased starch production, either by directly affecting the regulation of starch biosynthesis genes or by indirectly altering the processes that govern glucose production. The observation of starch accumulation during infection has already led to new methods for HLB detection (6, 7), while a more advanced understanding may help identify novel targets for symptom alleviation and/or disease control.

To date, the existence of a functional ATP/ADP translocase in a facultative intracellular bacterium has not been reported. It has been suggested that ATP/ADP translocases exist in obligate intracellular bacteria and not facultative intracellular bacteria because the risk of ATP exportation resulting from the system may be too great in an extracellular environment (4). Since the nature of the relationship between the bacterium and the insect is not currently known, this line of logic implies that the life cycle of "*Ca.* Liberibacter asiaticus" within the insect host may also be obligate in nature. However, it is interesting that upon completion of the genome sequence of the nonintracellular bacterium *Xylella fastidiosa*, a gene showing 22% identity and 38% similarity to an ATP/ADP translocase gene was documented, although this gene was never functionally characterized (17). Considering that only moderate

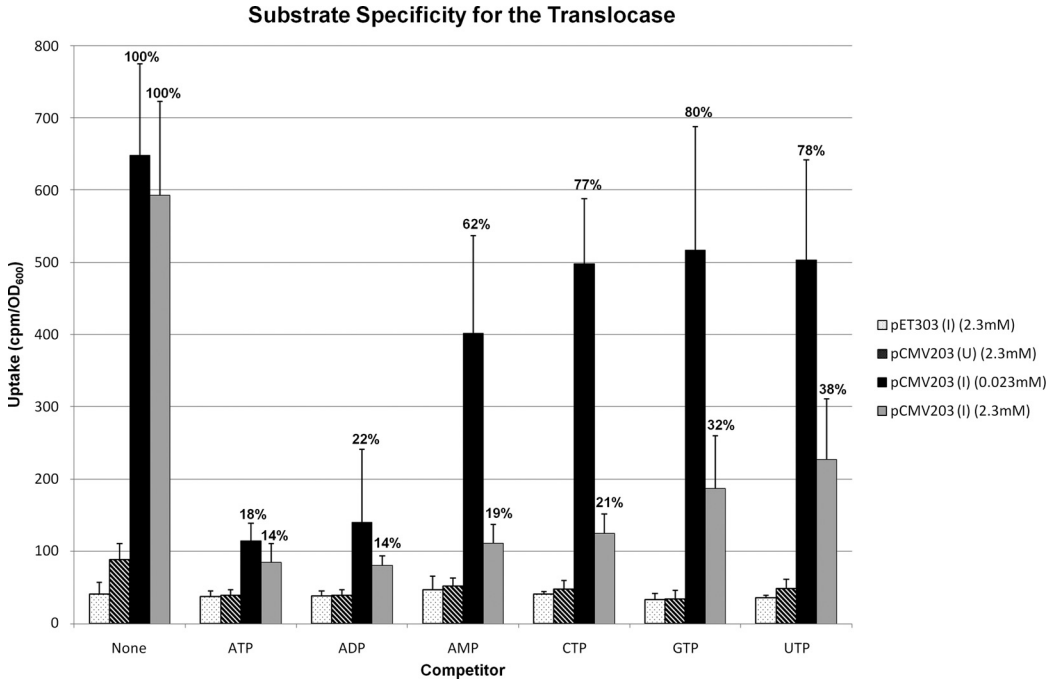

FIG. 4. Results from a competition assay with nonradiolabeled nucleotides. Cultures containing an inducible plasmid carrying *nttA* (pCMV203) or the vector alone (pET303) were grown at 37°C. IPTG was added to induce *nttA* expression, and both uninduced (U) and induced (I) cultures were grown for an additional 60 min. Aliquots were removed and resuspended in PBS containing [α-^{32}P]ATP. The substrate specificity of NttA was determined by adding unlabeled nucleotides at a concentration of 0.023 or 2.3 mM to the reaction mixture and incubating the mixture at 30°C for 20 min. Samples were washed and measured on a scintillation counter. The average amount of radioactive-ATP uptake (expressed as counts per minute per unit of OD_{600}) in three independent experiments was calculated, and the standard deviation is shown. The percentages of uptake at the two different nucleotide concentrations compared to the levels of uptake in the corresponding samples that contained no unlabeled nucleotides are listed above the error bars.

amounts of sequence identity exist among proteins determined to be members of the translocase family, it is possible that *X. fastidiosa* may be an example of a nonintracellular bacterium encoding a translocase. Further investigation is necessary in order to properly identify the function of this putative gene in *X. fastidiosa*.

Overall, functional ATP translocases have now been identified in both plant and animal pathogens. Since the mechanism of ATP uptake appears to be conserved, an evolutionary relationship among bacteria containing an ATP translocase may also exist. Analysis of the phylogenetic relationships among the translocases from "*Ca*. Liberibacter" bacteria and other known translocases may aid in our understanding of the evolution of these important plant pathogens.

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Las  MSEAKKFIPMTLMMFLVLFNFSTLRPIKDSLVVPHMGAEIISFVKLWCVFPSAILF
Lso  -------------------------L-------N----V----------------

Las  TILYMKLSNILETEKLFYVVVSFFVSFFAIFAFLLYPYRDFLHPSPESIRALASSW
Lso  --I--------DS-----I---------V----V-------I----QT-KIWV--F

Las  PYLKWFIYLAGKWSFALFYVFAELWGAVMINLMFWQFANRITKTEEATRFYATFGL
Lso  -FF---LF-------------------------------------------------

Las  VGNVGLIFAGGIVKTFSKLTNELMIYYSMLTLILSSILLMAVYRWMNLYVLTDPQQ
Lso  ------------IIKL----DA--------------F----I-----V------K-

Las  YSPKRGSVKKKLKLSVLESLRMALNSKYLGYLVLLVICYGTAINLVEGPWKEKVRQ
Lso  ---RK-AL--------SD--K----------I-------------------A---E

Las  LYPTQNSYAQFMGQFIQWTGIVTIVFMILGSNILKSFRWFTSAIITPLMILITGGG
Lso  ---S--D-----------------------------VS------------------

Las  FFVFVIFEDALLPFTEDVIKLVPLALAVFLGSLQNVLSKATKYTMFDSTKEMAFIP
Lso  --------N----L---IM--T--V------A---I--------------------

Las  LDDEFRTKGKAVVDVIGGRMAKSGGAVIQSFIFMLFPMATFSSIVPYLAVFFLCLM
Lso  -----KI------------L--------------FV------T-I-F-TGI--F-

Las  FIWIYVVNQLSKEYTQLISKE
Lso  -M-----T--N------LD--
```

FIG. 5. Identification of a putative ATP translocase in "*Ca.* Liberibacter solanacearum." A BLAST search revealed the presence of a gene encoding a protein similar to NttA in the genome of the "*Ca.* Liberibacter" species known as "*Ca.* Liberibacter solanacearum." The schematic shows an amino acid alignment of the translocase from "*Ca.* Liberibacter asiaticus" (Las) with that from "*Ca.* Liberibacter solanacearum" (Lso). Dashes represent amino acids that are identical, while those that differ are shown in black. The 12 transmembrane domains are highlighted in gray.

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